chloride (ACh) 0.3 M, pH 4.0–5.0, monosodium glutamate 0.25 M, pH 8.0–9.0, nicotine 0.2 M, pH 3.0–4.0, dihydro-β-erythroidine (DHβE) 10 mM (in 0.165 M NaCl), pH 4.0–5.0, acetyl-β-methylcholine chloride (methacholine) 0.5 M, pH 4.0–5.0, atropine Sulphate 30–40 mM, pH 5.0–6.0 and muscarine chloride 0.3 M, pH 4.0–5.0. Pontamine sky blue was used for current control and marking neurones as previously described (Boakes, Bramwell, Briggs, Candy & Tempesta, 1974). The neurones tested were concentrated in the area of nucleus ambiguous and the lateral reticular nucleus.

Most neurones tested were excited by acetylcholine which increased both the number of spikes per burst and the number of bursts per epoch. This effect contrasts with the glutamate response at higher currents where the discharge pattern changed from phasic to tonic. Nicotine excited the majority of neurones. Sometimes the effect of nicotine was prolonged as was seen when the drug was applied to non-respiratory cells, but more often a more rapid effect was obtained similar to that seen with acetylcholine on respiratory cells.

All nicotine responses were blocked by DHβE at very low currents. Acetylcholine responses were abolished in a few cases but even when ACh was applied at low currents were more resistant to the antagonist than were nicotine responses. Atropine was applied to a few neurones and blocked the acetylcholine response in all cases. Muscarine excited most cells tested but the muscarine agonist methacholine even at high currents produced little effect.

From the data obtained so far it appears that there are not separate populations of respiratory cells displaying either muscarinic or nicotinic sensitivity. Instead the evidence points to both muscarinic and nicotinic receptors being present on the same neurone.

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Further evidence for the possible co-existence of 5-hydroxytryptamine and substance P in medullary raphe neurones of rat brain

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Recent results have indicated that certain medullary raphe neurones that accumulate radioactive 5-hydroxytryptamine (5-HT) (Chan-Palay, Jonsson & Palay, 1978), and contain immunoreactive 5-HT (Hökfelt, Ljungdahl, Steinbusch, Verhofstad, Nilsson, Brodin, Pernow & Goldstein, 1978), also contain substance P-like immunoreactivity. In order to investigate this possibility further, we examined the effects of the neurotoxin 5,6-dihydroxytryptamine (5,6-DHT) on descending substance P (SP) fibres in rat spinal cord.

Rats received a single injection into the lateral ventricle of 5,6-DHT (75 μg free base) dissolved in saline containing ascorbic acid 0.5 mg/ml. Examination of the SP content of spinal cord and ventromedial medulla revealed significant SP depletions (Table 1) 2 weeks after 5,6-DHT in areas previously shown to be almost completely depleted of 5-HT following 5,6-DHT treatment, (Baumgarten, Björklund, Lachenmayer, Nobin & Stenevi, 1971). The dose of 5,6-DHT used does not deplete spinal cord noradrenaline (NA) and in fact after 10 days NA levels are raised above control levels (Nobin, Baumgarten, Björklund, Lachenmayer & Stenevi, 1973). There was only a small depletion of SP from the medulla and the most rostral parts of the spinal cord (14–37%), but there was a marked reduction of SP in the ventral spinal cord, especially in lumbar segments (>90%). There was also a smaller, but significant, loss from dorsal spinal cord (26–46%). At longer times after 5,6-DHT (up to 20 months) there was no recovery of SP content in any region of spinal cord or medulla, except the region containing the cell bodies of the medullary raphe nuclei. Immunohistochemistry 2–3 days after the administration of 5,6-DHT revealed accumula-

tions of SP-immunoreactivity in medullary raphe neurones, and distorted, presumably degenerating, axons containing SP in the medulla and spinal cord. Immunohistochemical studies at longer times after 5,6-DHT (up to 20 months) failed to show any reappearance of SP-containing fibres or terminals in areas depleted by the toxin treatment. This suggests that the neurones which may contain both SP and 5-HT represent a separate population of raphe neurones, since other 5-HT cells in the medullary raphe have been shown to sprout extensively after 5,6-DHT and reinnervate many of the denervated areas (Baumgarten, Björklund, Lachenmayer, Rensch & Rosengen, 1974).

The possible descending SP/5-HT system revealed by 5,6-DHT injection may correspond to the pathway known to produce supraspinal analgesia (Basbaum, Clanton & Fields, 1976).

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Table 1 Effect of 5, 6-DHT on substance P content of medulla and spinal cord

	Substance P content (ng/g protein)		
Region	*Control*	*2 weeks*	*20 months*
Medullary raphe	699 ± 56 (3)	*440 ± 67 (3) (−37%)	703 ± 128 (4) (+0.6%)
Dorsal cervical Spinal cord	3109 ± 292	*1675 ± 192 (3) (−46%)	*1261 ± 281 (4) (−59%)
Ventral cervical Spinal cord	802 ± 161 (3)	693 ± 63 (3) (−14%)	*427 ± 88 (4) (−47%)
Dorsal lumbar Spinal cord	2909 ± 110 (3)	2148 ± 764 (3) (−26%)	*1604 ± 109 (4) (−45%)
Ventral lumbar Spinal cord	704 ± 48 (3)	*49 ± 45 (3) (−93%)	*62 ± 19 (4) (−91%)

SP contents of tissue samples were measured by a radioimmunoassay (Kanazawa & Jessell, 1976).
Values in brackets represent numbers of animals used for each determination, and percentage change in SP levels from control.
*Denotes $P<0.05$ by Students t-test.

Dose dependent behavioural stimulation after local infusion of substance P into the ventral tegmental area in the rat

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Evidence is accumulating that the polypeptide substance P (SP) is an excitatory neurotransmitter in the mammalian CNS. Regional distribution studies have revealed that particularly high concentrations of SP are found in the ventral mesencephalon. One SP-containing pathway which has been described in detail originates in the medial habenula and innervates the ventral tegmental area (VTA) (Cuello, Emson, Paxinos & Jessell, 1978). The VTA contains the dopaminergic A 10 (DA-A10) cell bodies which form